



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

A STUDY OF STREPTOCOCCI FROM MILK AND FROM EPIDEMIC SORE THROAT, AND THE EFFECT OF MILK ON STREPTOCOCCI.*

E. C. ROSENOW.

(*From the Memorial Institute for Infectious Diseases, Chicago.*)

During the recent epidemic of sore throat in Chicago, shown by Capps and Miller¹ to have been spread by milk, I had the opportunity to study streptococci from numerous throats, from the pus of suppurating glands, and from exudates in a number of fatal cases. In a joint note with Dr. D. J. Davis² it was pointed out that these streptococci possessed certain peculiarities: They appeared mostly as diplococci and short chains surrounded by a capsule; their growth on blood agar was not mucoid, but more abundant and with less hemolysis than that of the typical streptococcus pyogenes, from which they, however, did not differ in fermentative properties. In no instance was inulin fermented. For a more complete description see the paper by Dr. Davis and myself and the recent paper by Davis.³ It was found that these properties varied according to the severity of the infection. Many cases of ordinary tonsillitis yielded the ordinary streptococcus pyogenes. The special features mentioned were most marked in fatal cases, and the strains isolated from the peritoneal exudate and blood would show them to a greater degree than the ones isolated earlier in the attack or at the same time from the tonsils. After cultivation on blood agar it was noted that the strains from the tonsils soon lost any distinctive peculiarities, whereas those from the exudates in the fatal cases retained them longer. This was true not only of the strains from different cases but also of the strains from the same case. Thus, in four instances the throat strains lost the special properties in from one to four weeks, whereas two of the strains from the exudates were still growing in the original manner, though not so marked, after six and seven months. Hence it is clear that the cases were due to streptococci with distinctive morphological and

* Received for publication September 1, 1912.

¹ *Jour. Am. Med. Assoc.*, 1912, 58, p. 1848.

² *Ibid.*, p. 773.

³ *Ibid.*, p. 1852.

cultural features, and, furthermore, that as a result of growth in the fluids of the body these features may become accentuated and more permanent.

The epidemics in Boston¹ and in Baltimore² were of the same general nature as the one in Chicago, being milk-borne, and associated with similar streptococci. It should be noted that each epidemic occurred during the winter or early spring, seasons when sore throats and tonsillitis are common. In other milk-borne epidemics, in which blood agar methods were not used in the study of the streptococci, it is impossible to say whether the streptococci resembled the ones described by us.

The source of the streptococci in milk-borne epidemics of sore throat, whether from diseased udders or from the throats of milkers or others handling the milk, has been discussed a great deal. Davis³ points out that during the epidemic in Chicago there existed an unusually large number of cases of mastitis in the cows which furnished the incriminated milk, and that tonsillitis among the milkers was also common. Most observers now hold that the streptococci found in milk cannot be differentiated clearly, culturally or morphologically, from the ordinary streptococcus pyogenes. Puppel⁴ and Ruediger,⁵ however, believe that this may be done by means of blood agar plates, as the milk streptococci do not cause hemolysis while the streptococcus pyogenes does. Gminder⁶ has shown that streptococci from chronic mastitis in cows are of little virulence for rodents but produce mastitis in goats. Puppel is also inclined not to attribute much importance to the streptococci in mastitis, and Heinemann,⁷ by animal passage, succeeded in making the milk streptococcus as virulent as the streptococcus pyogenes. Notwithstanding the recent evidence that milk not infrequently spreads streptococcus sore throat, the attempts to isolate virulent streptococci from milk have usually resulted in failures. I shall now present certain results that I have obtained from the study of milk in relation to streptococci.

¹ Winslow, *Jour. Infect. Dis.*, 1912, 10, p. 73.

² Hamburger, *Jour. Am. Med. Assoc.*, 1912, 58, p. 1109.

³ *Loc. cit.*

⁴ *Ztschr. f. Hyg. u. Infektionskr.*, 1912, 70, p. 3.

⁶ *Centralbl. f. Bakt.*, I, Orig., 1912, 63, p. 152.

⁵ *Science*, 1912, 25, p. 223.

⁷ *Jour. Infect. Dis.*, 1907, 4, p. 89.

THE ISOLATION OF VIRULENT STREPTOCOCCI FROM "SEPARATOR SLIME" AND FROM MILK, CREAM, AND BUTTER.

In my work on experimental endocarditis I learned how harmless non-virulent bacteria are when injected intravenously in rabbits and guinea-pigs, and how rapidly their destruction takes place. It was thought that this method might serve to separate avirulent from virulent streptococci and other bacteria in milk.

There is being introduced into the dairy industry by the De Laval Separator Company a clarifying machine. The "slime" obtained in this way and also from the bowl of the ordinary cream separator contains enormous numbers of streptococci and other bacteria, leukocytes, and foreign matter. This material seemed specially adapted for use in the tests to be made. The "slime" was collected in sterile bottles, packed in ice, and sent to the laboratory. Rather dense emulsions were made in NaCl solution, centrifugated fractionally, and the turbid supernatant fluid examined in various ways. Nine samples of "clarifier slime" and six samples of "separator slime" were examined. The former represented four central stations, the latter six individual dairies, some of the latter being separated by a distance of 400 miles. The samples at the time examined were from 24 to 72 hours old and all reacted acid to litmus. Rabbits were injected with from 6 to 15 c.c. of the suspension depending upon the size, guinea-pigs with from 5 to 7 c.c., while mice received from 0.5 to 1 c.c. In order to make sure that the results were not due in part to other material in the milk than bacteria, cultures were made in ascites meat broth, incubated for 12 to 18 hours, and then injected. Both methods gave the same results. The animals usually died of streptococemia in from 24 to 72 hours. Smears of the emulsions, cultures on blood agar plates, and broth cultures showed streptococci in greatly predominating numbers in all the samples. The next most common organism was what appeared as a pseudo-diphtheria bacillus. In order to test whether the strains isolated from the blood of the animals which died were really pathogenic, a second and in some instances a third inoculation of small size was made. Virulent streptococci were obtained after death of the animals from the blood or peritoneal exudate or from both in 13 of the 15 samples

injected. Intravenous injections in the rabbits gave the highest percentage of positive results. In most cases (24) streptococci in pure culture were obtained, streptococci in predominating numbers but mixed with colon bacillus in nine, and the colon bacillus pure in one. One animal yielded a pure culture of a typical pneumococcus. Nine animals survived while three developed streptococcus arthritis later. It should be stated that in the six dairies from which "slime" was obtained there were no cases of mastitis in the cows and no tonsillitis in the milkers, at least so far as was known. In the case of the central stations, these points could not be ascertained definitely. The blood agar plates showed hemolyzing colonies of streptococci in small numbers in only four samples; non-hemolyzing streptococci were obtained in large numbers in all. From one sample the plates yielded an organism which resembled morphologically and culturally (growing in symbiosis with streptococci) the influenza bacillus, but it was not found after death in the animals injected with this sample.

Virulent streptococci were obtained also from two samples each of milk and cream, obtained in the open market and pasteurized by the flash method. One of two samples of butter gave a similar result. The blood agar plates again showed the non-hemolyzing streptococci in large numbers, but no hemolyzing colonies. Milk pasteurized by the holding process yielded negative results.

The difference in the character of the growth of the streptococci on blood agar plates before and after animal passage was striking, hemolyzing colonies only being present on the plates from the blood of the animals and almost exclusively non-hemolyzing colonies on the plates made directly from the suspensions. This finding brings up the point whether the non-hemolyzing streptococci become hemolytic on animal passage or whether the virulent streptococci were present in such small numbers as to be missed in the first plates. The tests for virulence before animal passage of strains which came from single non-hemolyzing colonies and a number of hemolyzing colonies resulted negatively even though very large doses were injected. In view of this fact, and because of the total absence of non-hemolyzing colonies in the animals after death, it would seem that virulent streptococci were present in small num-

bers and hence missed in the plates from the original material. This seems to explain why virulent streptococci are obtained from milk so rarely by the plate method. Most of the strains of streptococci obtained from the animals appeared in diplococcus forms and as short chains, surrounded by a well defined capsule. Hemolysis was less marked about those colonies which were particularly large and moist and consisted of cocci with an abundance of capsular substance. These features were soon lost on artificial cultivation, the organisms now resembling the ordinary hemolytic streptococcus. One strain was passed through three successive guinea-pigs. It now grew exactly like *streptococcus mucosus*, had a wide capsule, and did not ferment inulin. This strain in particular as well as one other resembled very closely indeed those obtained from the throats during the epidemic of sore throat. This was believed not to be a mere coincidence, and it was decided to test the effect on streptococci of milk obtained in as sterile a manner as possible.

EXPERIMENTS WITH MILK OBTAINED IN STERILE FORM.

The following technic was used in an attempt to procure sterile milk directly from the cow. Milking tubes, well known in the dairy industry, were sterilized after being provided with a metal hood and a suitable container. The attempt was made in a model dairy where rigid cleanliness is enforced. The udder was washed with a solution of formalin; the teat to be used was milked approximately one-fourth empty and the mouth of the teat sterilized with 1-1,000 bichloride; the tube was inserted, a rather large amount of the milk allowed to escape, and then 200 c.c. of milk were collected under a hood in each of two bottles. Part of this was tubed at once, from 0.5 to 4 c.c. being placed in each tube. Plate cultures on plain and blood agar were made at once from the bottles to determine the number of bacteria present. Part of the samples were pasteurized (at 60° C. for 20 min.), part placed in the incubator, and part in the ice-chest. Five of 12 normal cows yielded sterile milk in this way. The number of colonies in the milk of the others per c.c. was as follows: 800, 4,000, 3,500, 400, 4, 14, and 9. Three of these showed streptococci in large numbers after the milk was incubated, and two of these when injected into

guinea-pigs proved virulent. The milk of one cow yielded a typical virulent pneumococcus. These three cows had not had any disease of the udder at any time. (The milk from two cows with chronic mastitis contained an enormous number of hemolyzing streptococci in pure culture, one loop yielding countless numbers of hemolyzing colonies on blood agar. Both proved only moderately virulent for rabbits, guinea-pigs, and white mice.)

The effect of the sterile milk on streptococci was now tested. First aerobic and anaerobic cultures on blood agar were made to determine whether the milk to be used was sterile. The tests were in duplicate. After inoculation with ordinary streptococci one set of tubes was placed at 37° C., the other kept at room temperature and part of the time in the ice-chest, thus imitating the conditions that exist in the routine handling of milk. It soon became apparent that the tubes placed at 37° C. and in which growth, with acid production, occurred did not appreciably change the streptococci, whereas the streptococci in the milk kept outside the incubator were modified perceptibly. Streaks with this milk on the surface of blood agar plates showed a more abundant growth and less hemolysis than did the streaks from the incubated milk. That the milk carried over with the loop was not the cause of this difference was shown by placing a loopful of culture from the surface of blood agar slants into milk and making the streaks at once. No change occurred.

The effect of whole milk was studied on 15 strains of virulent streptococci. Four of these strains were ordinary hemolytic streptococci. Five—all growing like typical hemolytic streptococci—came from the animals in the above experiments and six from cases of sore throat during the epidemic. Four of the latter had reverted to typical streptococci while two still grew in the peculiar manner. The strains were obtained originally from single colonies, but were plated out a second time and subcultures again made from a single colony in order so far as was possible to be sure that the results obtained might not be due to a mixture of strains.

The experiments with the following strain (595) illustrate in general the results obtained. This strain had been isolated from the throat of a case of scarlet fever nine months previously and had

since been cultivated continuously on blood agar. During this time it had no capsule and always produced small colonies surrounded by a wide zone of hemolysis. On May 9 a subculture was made on blood agar; on May 11 a culture was made from this on a blood agar slant and also two inoculations of sterile milk. The slant and one of the tubes of inoculated milk were placed at 37° C; the other tube of milk was kept at room temperature and in the ice-chest. On May 14 subcultures were made side by side from each of the tubes on blood agar plates, which were placed at 37° C. The plates from the blood agar slant and from the incubated milk tube (which had become strongly acid in reaction) gave only scant growth but with marked hemolysis; no capsules could be demonstrated in either case. The growth from the milk tube kept at a low temperature was more abundant. At the end of 15 hours there was no trace of hemolysis, at the end of 24 hours a rim one millimeter wide had formed which at the end of 48 hours was about two millimeters in width. The cocci were surrounded by a definite, eosin staining capsule. Subcultures on blood agar soon lost these features even though the inoculations were made with streptococci placed in a loop of milk just previously. On the other hand, alternate cultures for 24 hours on blood agar and in milk soon increased the capsular substance and reduced the hemolysis.

It was now determined to test the virulence of the two types. Both were inoculated into ascites broth and grown for 24 hours. The culture from blood agar showed rather long chains with no capsule, while those from the third generation in milk consisted chiefly of diplococci with only an occasional short chain, surrounded by a capsule. Diminishing but equivalent doses of each strain were injected intraperitoneally in mice, and all died at the end of 72 hours. Those which had received the milk-treated cocci died first, and their blood contained encapsulated cocci, while the blood of the mice injected with cocci from blood agar showed typical hemolytic cocci. Two guinea-pigs were injected subcutaneously with the blood agar strain, and survived without developing symptoms; those injected in the same way with the milk-treated strain developed a marked local reaction and arthritis, and died in 12 days. Cultures from the indurated area and from the blood yielded a pure culture of a streptococcus which resembled

those from the fatal cases in man; the joint cultures remained sterile.

Some of the throat strains, recently isolated, were modified even more strikingly by this treatment, whereas a highly virulent strain from the blood of a fatal case of bronchopneumonia in man was not influenced perceptibly by the milk.

Treatment with milk seemed to have a marked effect on strains from the fatal cases during the epidemic which had lost part of their original peculiarities. A single soaking in milk would bring back fully the former characteristics. This was true also of two strains from the Boston epidemic, now 18 months ago, kindly given me by Dr. D. J. Davis.

One of my strains, cultivated for six months, reverted to the original type on passing growths on blood agar through two guinea-pigs; similar passage of a strain grown in milk resulted in growths exactly resembling the streptococcus mucosus.

In this connection it should be mentioned that subcultures on blood agar plates from the upper and much-dried portion of old blood agar tube-cultures (72 days) of streptococci from fatal cases for several generations produced dry fine colonies surrounded by a well defined zone of hemolysis, while cultures from the lower and moist part of the same old tubes gave moist large colonies with little hemolysis. Concentration of salts during the evaporation of the agar would seem then to have an influence on the morphology and amount of hemolysis produced by the streptococci. This point should be studied further.

As unheated sterile milk has the most pronounced effect on streptococci, pasteurized milk a somewhat less pronounced effect, and autoclaved milk little or no effect, salt concentration cannot be the factor here. The effect of the milk is independent of the cream content. It is interesting that the modification should occur only when growth of streptococci is prevented or delayed by placing the mixtures at a low temperature.

A comparative study of the resistance to heat of various streptococcal strains has also been made. Suspensions from blood agar slants were made in broth and unheated sterile milk. These were then heated to 45° and 60° C. for 20 minutes respectively. The lower temperature of 45° C. was sufficient to sterilize the strains of

high virulence in the milk suspensions, whereas none were sterile of those suspended in broth. Most strains from blood agar plates, which were non-virulent, showed growth after heating to 60° C. for 20 minutes, whereas the virulent strains similarly treated all were killed.

SUMMARY.

Streptococci, virulent for animals, but which differ from typical streptococcus pyogenes in a more abundant growth, in being encapsulated and not forming chains, and in causing but little hemolysis, occur in predominating numbers in epidemic sore throat of milk-borne origin. On artificial cultivation these strains sooner or later assume the characteristics of streptococcus pyogenes.

Cultures on blood agar plates from ordinary milk usually give rise to colonies of streptococci that do not cause any hemolysis, but the injection of rabbits and other animals with milk "slime" practically always produces infection with encapsulated, but otherwise typical, hemolytic streptococci. The blood agar plate method consequently is not a reliable means with which to search for streptococcus pyogenes in milk.

By placing streptococcus pyogenes in unheated milk it becomes modified so as to correspond to the streptococci in epidemic sore throat. The modifications may be accentuated by passage through guinea-pigs, and in some cases cultures like those of streptococcus mucosus may result.

The fact that milk so modifies streptococci is an additional indication of the important part it may play in epidemic sore throat. It is not possible to determine whether the streptococci in such epidemics are of exclusively bovine or human origin; they may be of both.

Milk drawn in a sterile way from normal cows may contain virulent streptococci and pneumococci; hence "certified milk," while surely less contaminated than ordinary milk, may contain pathogenic bacteria, and the advisability of pasteurization even in this case should be considered, especially during seasons when sore throat is common.

Butter and cream may contain virulent streptococci.